To: Indiana State Department of Health

From: Eric Marcoux, Senior Quality Assurance Manager, RRGB

Cc: Ray Masters, Scott Schooler, Sharon Krull, RRGB

Date: August 10, 2009

Re: ROP HACCP Variance Request

Red Robin Gourmet Burgers executes a sealed-packaging process on its soups and sauces. The advantages in this process assists Red Robin with product portion control, facilitates quicker cooling, prevents potential cross contamination, provides a quicker and more thorough reheat application, and eliminates any further direct hand or utensil contact with product. Red Robin has executed this process for seven years successfully. Currently, Maricopa County in Arizona, the State of Virginia, and the state of New York have requested Red Robin apply for variance and all have approved and granted the variance.

Food safety and the well being of our Guests are a priority at Red Robin, and Red Robin will take every step necessary to assure the utmost diligence is taken in meeting all of the Department's requirements. Please accept the following information as Red Robin's submitted variance request and HACCP plan for the Department's review. Red Robin thanks the Department for their assistance and guidance that the Department has provided to assure the safety and health of Red Robin Guests.

Attached for your review is the information required per the Indiana State Department of Health – Retail Food Establishments Sanitation Requirements. Should you have any questions or if any further information is needed. Please feel free to contact me at 303-846-5480 or emarcoux@redrobin.com .

Red Robin Gourmet Burgers, Inc.
6312 S. Fiddler's Green Circle, Suite 200 North• Greenwood Village, CO 80111
(Phone) 303-846-5480 • (Fax) 720-493-2662 • emarcoux@redrobin.com

Request For Variance

State Form 51184 (12/02)
Food Protection Program

INDIANA STATE DEPARTMENT OF HEALTH
Telephone: 317/233-7360 FAX: 317/233-7334

1. Individual Submitting Request:

Date: 07 / 31 / 2009

Name: Red Robin Gourmet Burgers Telephone: (303) 846-5480 **Fax:** (720) 493-2662

Mailing Address: 6312 S. Fiddler's Green Circle Suite 200N (Number & Street) Email: emarcoux@redrobin.com

Greenwood Village, CO 80111
(P O Box) (City) (State) (Zip Code)

2. Person/Organization Seeking Variance:

Name: Eric Marcoux Email: emarcoux@redrobin.com

Mailing Address: 6312 S. Fiddler's Green Circle Suite 200N
(Number & Street)

Greenwood Village, CO 80111
(P O Box) (City) (State) (Zip Code)

3. Food Establishment(s) for Which Variance is Sought

Include the following information for each food establishment: (List here or attach additional pages if necessary)

- Physical Location (if different than mailing address): Red Robin Gourmet Burgers
- Mailing Address: See Attached locations listing
  (Number, Street, City, State, & Zip Code)
- Telephone Number: ( ) Fax Number: ( )
- Person at each retail food establishment most responsible for supervising: General Manager

4. State how the proposal varies from each rule requirement, citing relevant rule sections by number:
(Attach additional pages if necessary)
See attached

5. Explain how the potential public health hazards and/or nuisances will be alternatively addressed by the proposal. Include supporting studies, Hazard Analysis Critical Control Point (HACCP) Plan(s), standard sanitation operating procedures, and/or any other evidence: (Attach additional pages, if necessary.)
See attached

6. List how the proposal demonstrates the following (if applicable to the request):

A) How the proposal differs from what is common and usual in similar industry situations:
See attached

B) How the proposal is unique and not addressed in existing rules or law:
See attached

C) How the proposal does not diminish the protection of public health:
See attached

D) How the proposal is based on new scientific or technological principle(s):
See attached

E) How the implementation of the variance would be practical:
See attached

7. Explain how the person/organization seeking the variance will assure that all provisions of a granted variance will be enacted at each food establishment for which a variance has been granted:
See attached

8. List all affected parties known by the person/organization seeking a variance, including all affected regulatory authorities: (Attach additional pages if necessary)
Red Robin Gourmet Burgers
Indiana state Department of Health
Hamilton County
Vanderburgh County
Allen County
Lake County
St. Joseph County
Hendricks County
Porter County

9. Attach copies of any related variances, waivers or opinions issued by other governmental agencies.

For Office Use Only

10. Signature of Individual Making Request: _____

Printed Name, Title: Eric Marcoux, Senior Quality Assurance Manager, Red Robin Gourmet Burgers

Request for a Variance

Name of Variance Requested: Reduced Oxygen Packaging

Establishment Name: Red Robin Gourmet Burgers

Addresses:

6636 E Lloyd Expressway, Evansville, IN 47715 812-473-4100

9965 N. Michigan Road, Carmel, IN 46032 317-337-0183

14599 Clay Terrace Blvd, Carmel, IN 46032 317-574-0102

4201 Coldwater Rd., #27, Fort Wayne, IN 46805 260-484-9888

2465 Southlake Mall, Merrillville, IN 46410 219-736-7337

4010 N Main Street, Mishawaka, IN 46545 574-256-0100

13215 Harrell Parkway, Noblesville, IN 46060 317-773-8150

365 South Perry Road, Plainfield, IN 46168 317-839-2400

1410 US Hwy 41, Schererville, IN 46375 219-865-3900

3004 John Howell Dr., Valparaiso, IN 46385 219-531-7337

Contact Name: Eric Marcoux

Contact Address: 6312 S. Fiddler's Green Circle, Greenwood Village, CO 80111

Contact Phone: 303-846-5480

Relevant Code Sections Affected: 410 IAC 7-24-114; 410 IAC 7-24-195

Relevant Code Section Affected:

410 IAC 7-24-114 Variance Requirement

Red Robin Gourmet Burgers is responding to the Department's request to submit application for a variance from the Department for its chubbed soups and sauces procedure, as was determined by the Department as reduced oxygen packaging due to portioning hot soups and sauces in a sealed bag, and quick cooling the soups and sauces without reopening the bag prior to storing in the walk-in for later use.

The Department's identified potential hazard with the chubbing procedure is Clostridium botulinum. The Department, based on the FDA Model Food Code, explained that the chubbing procedure may produce an anaerobic environment in the sealed bag of 21% or less oxygen, which under this type of an environment may support the growth of C. botulinum.

Food safety, the well being of the guest, is a priority at Red Robin, and Red Robin will take every step necessary to ensure the utmost diligence is taken in meeting all the Departments requirements. Please accept the following information as Red Robin's submitted variance request and HACCP plan for the Department's review. Red Robin thanks the Department for their assistance and guidance that the Department has provided to ensure the safety and health of Red Robin guests.

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(Phone) 303-846-6069• (Fax) 303-846-6044• skrull@redrobin.com

Relevant Code Section Affected:

410 IAC 7-24-195 Reduced Oxygen Packaging, Criteria

Red Robin prepares four soup types and two sauces using the cook and/or chill procedure. Three additional items are not cooked and chilled, but portioned into a sealed bag, refrigerated and reheated. These items are marked with an asterisk. The menu items names are:

1) Chili
2) Clam Chowder Soup
3) French Onion Soup
4) Chicken Tortilla Soup
5) South West Pasta Sauce
6) Au Jus
7) Marinara Sauce*
8) Chipotle Beans*
9) Cheese Queso*

A HACCP plan for a Soups and Sauces category is included for the Department's review.

1. Method for maintaining food at 41° F or below is storing in the walk-in cooler, which maintains an ambient temperature of 41° F or below.

2. Each bag will display in bold, easily identifiable print, the required holding temperature of 41° F and to discard after 9 calendar days from pack.

3. The shelf life for all soups and sauces is 9 days from pack.

4. Product handling, operationally, does not include bare hand contact. It is dispensed by a utensil. Additionally, high temperature cooking and reheating provides a kill step for the presence of microbes.

5. There are no foods considered ready-to-eat in the soup and sauce recipes. A thorough high-temperature cook and reheat are performed prior to soups and sauces being served as ready-to-eat. At the time in which soup or sauce is a ready-to-eat food item, it is hot held, per regulation, and dispensed in a clean, sanitized bowl using a utensil.

6. Designated, trained Team Members only are permitted to use processing equipment. Team Members receive initial and ongoing training regarding food safety hazards and requirements to perform their tasks, as required per regulation.

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7. Food-contact surfaces, at minimum, are cleaned and sanitized as required by regulation. Almost all food-contact equipment is cleaned and sanitized using a commercial, approved dish machine, with wash, rinse, and sanitize steps per regulatory requirements. Larger or unusually sized equipment that is unable to fit in the dish machine are cleaned and sanitized using a three-step method (wash, rinse, and sanitize) in the approved, designated 3-compartment sink or clean in place.

8. Red Robin Team Member training includes, but is not limited to, the following training methods:

    a. Initial Team Member training for reduced oxygen packaging includes in-class knowledge and skills training, discussion, and demonstration.

    b. After the in-class training is conducted, Team Members move into the restaurant for an on-site demonstration by the specialized trainer while outlining the importance of each action step in relation to health and food safety. Next is for the Team Member to demonstrate the procedure, skills, and knowledge required while being coached and assessed by the specialized trainer. This is repeated until the Team Member's performance, skills, and knowledge are demonstrated to ensure health and food safety.

    c. Ongoing training is conducted on a daily basis through "Ready-Set-Gos and Ready-Set-Goodbyes," a Red Robin terminology for meeting with each Team prior to beginning and ending a shift to share concerns, performance issues, educate, etc.

    d. Continuous management and supervisory monitoring, coaching, quizzing and praising during the work shift regarding health and food safety.

    e. Corporate-based restaurant inspections and comprehensive food safety assessments are performed routinely to evaluate and further educate restaurant management and Team Members.

    f. Managers receive ServSafe certification.

    g. Written recipe procedures for quick reference with CCPs outlined.

Relevant Code Section Affected:

410 IAC 7-24-101 Variance and Waivers

Red Robin requests the Department to review the submitted information for the Department to issue a variance on Red Robin's chubbing procedure.

Relevant Code Section Affected:

410 IAC 7-24-115 Contents of a HACCP plan

(A) For the purpose of this variance request, soups and sauces that are chubbed in a sealed package are the potentially hazardous food category of concern.

(B) (1) Refer to the attached flow diagram specific to the "soup, sauces" category.
(2) Refer to the attached soup, sauces recipes that outline procedural control measures.

(C) Refer to pages 3 and 4 of this document for Team Member and supervisory training.

(D) Refer to the attached written HACCP plan.

(E) At this time, the Department has not requested additional scientific data or additional information.

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HACCP Plan

Product Description

Product Category/Description: Soups and Sauces

Common Name: Chili, French Onion Soup, Clam Chowder Soup, Chicken Tortilla Soup, Chipotle Beans, Marinara Sauce, Cheese Queso, South West Pasta Sauce, Au Jus

How is it to be Used: Served hot, at or above 140° F, to the Red Robin Guest

Type of Package: Prepared and portioned into sealed chub bags. Served in bowl.

Probable Consumer: Adults. Adolescents. Possibly Children. No highly susceptible populations, as defined by regulation.

Length of Shelf Life: Maximum 9 days from pack

Labeling Instructions: Chub bags will be identified with a maintain at 41° F cold holding temperature and discard after 9 calendar days, with the pack date and expiration/discard date noted.

Safe Handling Temperatures: Soups and sauces are cooked to 176° F for 10 minutes and reheated to an internal temperature of 165° F within two hours. Soups and sauce are quick cooled in an ice bath to 70° F within two hours, and to 41° F within four hours. Soups, sauces are held at 41° F for no more than 9-days in the walk-in. Soups and sauce are hot held at or above 140° F in a steam table.

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HACCP Plan

Product and Ingredient Identification

Category: Soups and Sauces

(* indicates cold holding, + indicates PHF)

Chili:

Ground beef patties*+	Chili Powder	Salt	Red Robin Seasoning
Black Pepper	Onion	Peppers	Minced garlic in water*
Flour	Tomato	Tomato Sauce	Black Beans
Beef Stock Base	Water		

Clam Chowder Soup:

Chowder Base, canned	Canned Clams	Milk*+	Whipping Cream*+
Dry White Sauce Mix	Worcestershire	Tabasco Sauce	

French Onion Soup:

Margarine*	Onions	Beef Stock Base	Water

Chicken Tortilla Soup:

Manufacturer Pre-made Tortilla Soup Base*+	Chicken*+	Water

Chipotle Beans (no cooking step. Seal bag, refrigerate, reheat only)

Black Beans	Chipotle Peppers	Salsa	Sugar	Seasoning

Marinara Sauce (Portioning only. Seal bag, refrigerate, reheat only)

Manufacturer prepared marinara sauce

Cheese Queso (Portioning only. Seal bag, refrigerate, reheat only):

Manufacturer prepared cheese Queso

South West Pasta Sauce:

Butter	Salsa	Parmesan cheese	Whipping Cream*+	Mexican Spice blend

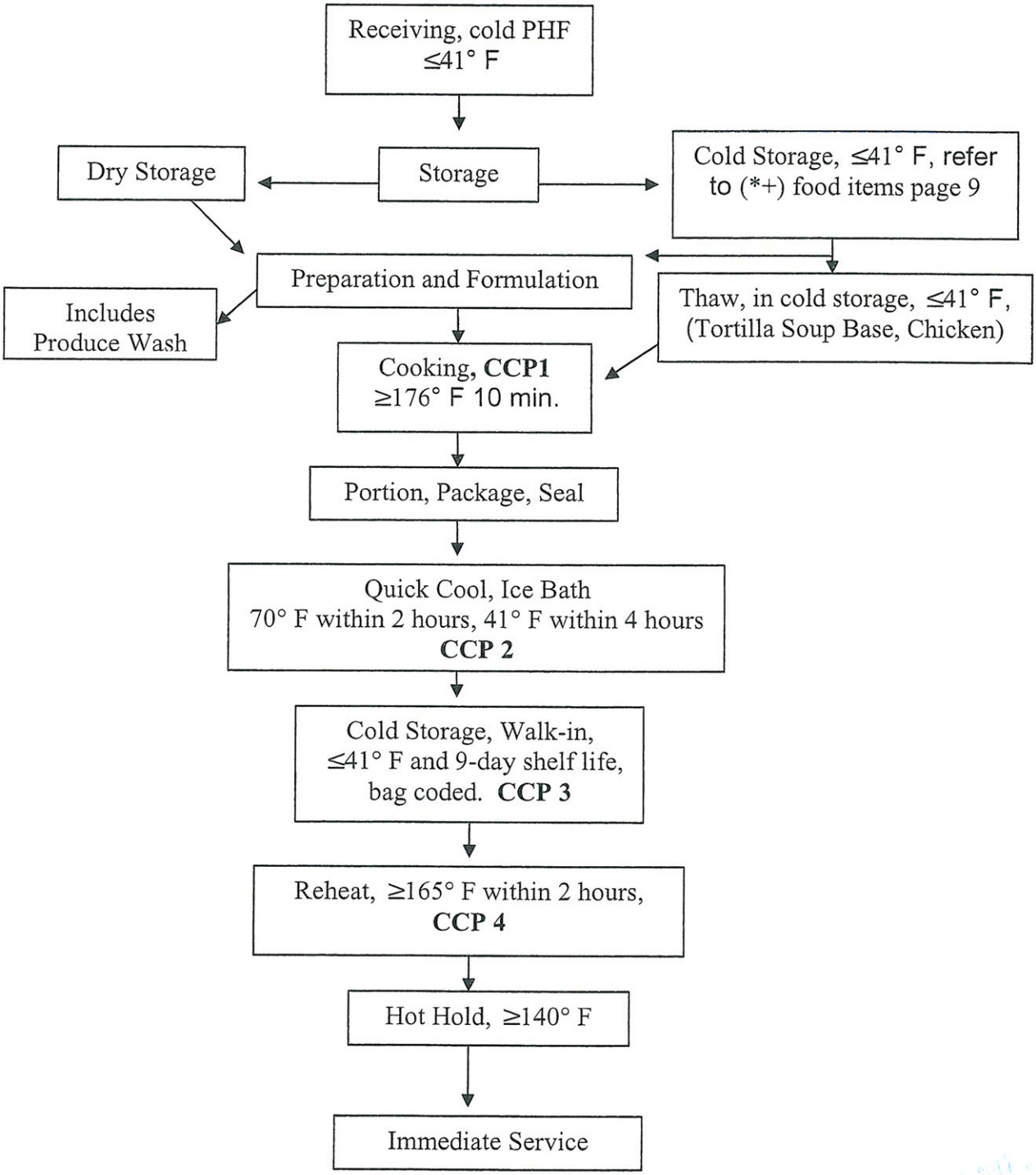
Au Jus:

Beef Stock Base	Water

Chubbing HACCP Plan

Flow Diagram

Category: Soups and Sauces



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Chubbing HACCP Plan

Hazard Analysis/Preventive Measures

Category: Soups and Sauces

Process Step	Hazards: biological, chemical, physical	In the absence of controls, is the hazard reasonably likely to occur? No: Stop Yes: Continue	Preventive Measures
Cooking	Biological: Pathogens Chemical: Deleterious Physical: Metal, glass	B: Yes, microbial kill step C: No, RRGB SOP for chemical storage, usage prevents likelihood of occurrence P: No, objects falling into food during cooking unlikely to occur due to RRGB equipment maintenance, no glass objects stored above food preparation areas, no storage capacity above steam kettle areas.	Temperature Control CCP 1
Cooling	Biological: Pathogens Chemical: Deleterious Physical: Metal, glass	B: Yes, Per FDA Food Code, cooked-chilled sealed bags create anaerobic environment for C. botulinum. Improper cool time and temperature promotes microbial growth. C: No allowable chemical entry into sealed bag. Supplier letters of guarantee for packaging. P: No allowable entry into sealed bag.	Temperature Control CCP 2

Chubbing HACCP Plan for C. botulinum

Hazard Analysis/Preventive Measures

Category: Soups and Sauces

Process Step	Hazards: biological, chemical, physical	In the absence of controls, is the hazard reasonably likely to occur? No: Stop Yes: Continue	Preventive Measures
Storage	Biological: Pathogens Chemical: Deleterious Physical: Metal, glass	B: Yes, PHF held at improper temperatures, and for extended shelf life promote microbial growth. C: No allowable entry into bag. P: No allowable entry into bag.	Temperature Control and Shelf Life CCP 3
Reheat	Biological: Pathogens Chemical: Deleterious Physical: Metal, glass	B: Yes, additional kill step for any microbial growth C: No allowable entry into bag. P: No allowable entry into bag.	Temperature Control CCP 4

Chubbing HAACP Plan
Hazard Analysis Critical Control Point Plan

Category: Soups and Sauces

Process Step/CCP	Hazard Description: biological, chemical, physical	Critical Limits	Monitoring Procedures, Frequency, Person Responsible	Corrective, Preventive Action, Person Responsible	HACCP Records	Verification of Procedures, Person Responsible
Cooking CCP 1	Biological—Bacteria, Clostridium botulinum, Viruses	Per RRGB, cook to internal temperature of 176° F for 10 minutes.	Designated, trained TM will take internal product temperatures of each batch during cooking until product reaches 176° F for 10 min.	Batches below 176° F for 10 min. will be reheated to 176° F within two hours and held for 10 min. Product not properly reheated for over four hours will be discarded by a manager.	Cook/Chill Record	Manager will observe cooking procedures once weekly, records verification.
Cooling CCP 2	Biological—Clostridium botulinum	Cool down to 70° F within two hours, and from 70° F to 41° F within four hours.	Designated, trained TM will take product temperature on a minimum of one bag per batch by folding bag and placing thermometer in center of fold.	Product not cooled within the required time period, critical limit, will be discarded by manager.	Cook/Chill Record	Manager will observe cooling procedure once weekly, records verification.

Chubbing HAACP Plan
Hazard Analysis Critical Control Point Plan

Category: Soups and Sauces

Process Step/CCP	Hazard Description: biological, chemical, physical	Critical Limits	Monitoring Procedures, Frequency, Person Responsible	Corrective, Preventive Action, Person Responsible	HACCP Records	Verification of Procedures, Person Responsible
Storage CCP 3	Biological—Clostridium botulinum	Store at ≤41° F, not to exceed beyond 9-day shelf life from pack.	Twice daily ambient storage cooler temperatures are taken and recorded by designated, trained RRGB TMs	PHF above critical limit for more than 4 hours will be discarded by manager. PHF above critical limits for less than 4 hours, will be quick cooled to ≤41° F or reheated to 165° F for service by Manager.	Cook/Chill Record	Manager will verify walk-in cooler temp daily. Observe shelf life weekly, records verification.
Reheat CCP 4	Biological—bacteria	Reheat to an internal temperature of 165° F within two hours.	Designated, trained TM will take internal product temperature on a minimum of one bag per batch by folding bag over and placing thermometer in center of fold.	Product not reheated within the required time period, critical limit, will be discarded by manager	Cook/Chill Record, includes thermometer calibration	Daily calibrated thermometer verified by manager Manager will observe reheat once weekly, records verification.

HAACP Cooking / Cooling / Cold Storage Chart 6/09

Period: _____ Week: _____
GM and KM Weekly Verification: Initials _____ Date: _____

Tortilla Soup

Date Prepped	Start Cooking Temp/Time at 176° F	Finish Cooking Temp/Time at 176° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

French Onion

Date Prepped	Start Cooking Temp/Time at 176° F	Finish Cooking Temp/Time at 176° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

Au Jus

Date Prepped	Start Cooking Temp/Time at 195° F	Finish Cooking Temp/Time at 195° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

Clam Chowder

Date Prepped	Start Cooking Temp/Time at 176° F	Finish Cooking Temp/Time at 176° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

Chili

Date Prepped	Start Cooking Temp/Time at 176° F	Finish Cooking Temp/Time at 176° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

Chipotle Beans

Date Prepped	Start Cooking Temp/Time at 176° F	Finish Cooking Temp/Time at 176° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

SW Pasta Sauce

Date Prepped	Start Cooking Temp/Time at 176° F	Finish Cooking Temp/Time at 176° F	Cooling Start time/temp	Cooling Time/Temp at 70° F	Cooling Time/Temp at 41° F	Shelf life ≤ 9 days at ≤ 41° F Yes or No

Corrective Action (if CCP is not met, complete the following):

CCP failure noted and food item:

Date and time failure occurred:

Corrective action taken, date and time action was taken:

Manager verification: Initials _____

HAACP Reheating and Hot Holding Chart 6/09

Period:_____ Week:_____

GM and KM weekly verification: Initials _____ Date _____

Tortilla Soup

Heat to 165 f - Hold at 150 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Clam Chowder

Heat to 165 f - Hold at 150 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

French Onion

Heat to 175 f - Hold at 170 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Chili

Heat to 165 f - Hold at 150 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Pasta Sauce (marinara)

Heat to 165 f - Hold at 150 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Bueno Queso (3 hr. shelf life)

Heat to 165 f - Hold at 150 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time
			Not Applicable	
			Not Applicable	
			Not Applicable	
			Not Applicable	
			Not Applicable	
			Not Applicable	
			Not Applicable	

Chipotle Beans (3 hr. shelf life)

Heat to 165 f - Hold at 150 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Au Jus

Heat to 195 f - Hold at 190 f

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Garlic Parmesan Spread (4 hr. shelf life)

Heat to 165 f - Hold at room temperature

Date Prepped	Reheat Start Time	Temp at 45 Minutes	Temp when placed onto Steam table	Expiration Time

Corrective Action (if CCP is not met, complete the following):

CCP failure noted and food item:

Date and time failure occurred:

Corrective action taken, date and time action was taken:

Manager verification: Initials _____ Date _____ Time _____

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0099-2240/96/$04.00+0

Growth of and Toxin Production by Nonproteolytic *Clostridium botulinum* in Cooked Puréed Vegetables at Refrigeration Temperatures

FRÉDÉRIC CARLIN* AND MICHAEL W. PECK
Institute of Food Research, Norwich, United Kingdom

Received 17 January 1996/Accepted 15 May 1996

Seven strains of nonproteolytic *Clostridium botulinum* (types B, E, and F) were each inoculated into a range of anaerobic cooked puréed vegetables. After incubation at 10°C for 15 to 60 days, all seven strains formed toxin in mushrooms, five did so in broccoli, four did so in cauliflower, three did so in asparagus, and one did so in kale. Growth kinetics of nonproteolytic *C. botulinum* type B in cooked mushrooms, cauliflower, and potatoes were determined at 16, 10, 8, and 5°C. Growth and toxin production occurred in cooked cauliflower and mushrooms at all temperatures and in potatoes at 16 and 8°C. The *C. botulinum* neurotoxin was detected within 3 to 5 days at 16°C, 11 to 13 days at 10°C, 10 to 34 days at 8°C, and 17 to 20 days at 5°C.

Six physiologically and phylogenetically distinct groups of clostridia are capable of producing the botulinum neurotoxin (18). *Clostridium botulinum* groups I and II are responsible for human foodborne botulism. *C. botulinum* group II (nonproteolytic) strains are psychrotrophic, produce toxins of types B, E, or F, and are capable of growth and toxin production at 3.3°C (18). Thus, nonproteolytic strains of *C. botulinum* pose a hazard in products that rely on a mild heat treatment and refrigeration for preservation, e.g., sous-vide products and other refrigerated processed foods of extended durability (REPFEDs) (26, 27). Vegetables are frequent ingredients of REPFEDs, and cooked vegetables are considered a high risk with regard to nonproteolytic psychrotrophic *C. botulinum* (2). A wide range of cooked vegetables incubated at 30°C supported growth and toxin production by a mixture of strains of nonproteolytic *C. botulinum* (8). Nevertheless, there is little information on the growth of nonproteolytic *C. botulinum* in cooked vegetables at refrigeration temperatures and under conditions of mild temperature abuse. In contrast, growth of and toxin production by nonproteolytic *C. botulinum* in laboratory media (16, 17, 22, 25, 28), in meat and poultry (1, 14, 29, 30), and in fish and seafood (6, 7, 12, 13, 19–21, 23, 24, 36, 39, 40) have been extensively studied.

The aim of this work was (i) to determine the ability of each of seven strains of nonproteolytic *C. botulinum* to grow and produce toxin in a range of cooked vegetables at mild abuse and refrigeration temperatures and (ii) to study the effect of temperature ranging from 5 to 16°C on the kinetics of growth of and toxin production by nonproteolytic *C. botulinum* type B in selected vegetables.

Growth of and toxin production by different strains of nonproteolytic *C. botulinum* in a range of vegetables. Cooked purées were prepared from fresh vegetables, distributed anaerobically in 10-ml volumes, and sterilized as described previously (8). The strains used were nonproteolytic *C. botulinum* type B (Eklund 2B, Eklund 17B, and Hobbs FT50), type E (Beluga and Hazen 36208), and type F (Eklund 202F and Craig 610). The origin and maintenance of the strains and the preparation of spore suspensions were as described previously (8, 34). Suspensions of spores of each strain were diluted in sterile glass-distilled water to give a concentration of 10^5 viable spores per ml. A 100-μl sample of each spore suspension was inoculated individually into two to four replicate vials containing 10 ml of cooked puréed vegetables to give a final concentration of 10^3 spores per ml. In some cases, tests were performed with different batches of cooked vegetables. Vials were incubated for 15 to 60 days at 30 and 10°C. Vials were observed daily for production of gas bubbles for the first 15 days of incubation and then every 3 to 4 days. On the final day of incubation, samples were tested for toxin by an enzyme-linked immunosorbent assay (ELISA) method (8, 37), and in some cases, the mouse test was also used (35).

At 30°C, growth and/or toxin production by all strains was detected in mushrooms, potatoes, and cauliflower, as well as by five strains in broccoli, four strains in asparagus, and two strains in kale (data not shown). Cooked bean sprouts supported the growth of strain 17B, the only strain tested. At 10°C, visible growth (gas production) was detected in mushrooms within 7 days of incubation with six of the inoculated strains, and production of toxin was confirmed (Table 1). The presence of the botulinum neurotoxin was demonstrated in inoculated vials of mushrooms, cauliflower, broccoli, asparagus, and kale with an ELISA and the mouse test (Table 1). In the ELISA, most positive samples gave an A_{492} substantially greater than the threshold for a positive ELISA as calculated by Potter et al. (37), indicating the production of a significant amount of toxin.

This work has confirmed the ability of nonproteolytic *C. botulinum* to grow and produce toxin in a range of cooked vegetables, such as mushrooms, cauliflower, potatoes, broccoli, asparagus, bean sprouts, and kale at 30°C (8). Some vegetables, such as mushrooms and cauliflower, supported growth of all strains tested, while fewer strains grew on other vegetables (e.g., kale). Growth and toxin production have now also been demonstrated at refrigeration temperatures. Five vegetables that supported growth at 30°C supported the growth of at least one strain at 10°C. This article is the first report of toxin production at 10°C by nonproteolytic *C. botulinum* in asparagus, broccoli, cauliflower, kale, and mushrooms; toxin production has also been previously reported in potatoes (5, 31) and peas (15).

* Corresponding author. Present address: Institut National de la Recherche Agronomique, Station de Technologie des Produits Végétaux, Domaine Saint Paul, Site Agroparc, 84914 Avignon Cedex 9, France. Phone: (33)90316000. Fax: (33)90316258. Electronic mail address: FREDERIC.CARLIN@AVIGNON.INRA.FR.

TABLE 1. Growth of and toxin production by strains of nonproteolytic *C. botulinum* in vials of cooked puréed vegetables incubated at 10°C[a]

Vegetable or medium	pH	Total incubation time (days)	Result for nonproteolytic *C. botulinum* test strain													
			Hobbs FT50		Eklund 17B		Eklund 2B		Beluga		Hazen 36208		Eklund 202F		Craig 610	
			TVG[b]	Toxin[c]	TVG	Toxin	TVG	Toxin	TVG	Toxin	TVG	Toxin	TVG	Toxin	TVG	Toxin
PYGS	6.86	15	5–9	+	6–10	+	6–9	+	5	+	6	+	5	+	5	+
Mushroom	6.41	15[d]	5–7	++	6–7	++	7–8	++	6–12	++	GNO	++	5	++	5–6	++
Cauliflower	5.47	60	GNO	+*	GNO	++	GNO	−	GNO	−	GNO	−	GNO	++	23	+*
Broccoli	5.49	60	19–23	++	GNO	++	GNO	++	GNO	−	GNO	−	GNO	++	GNO	++
Asparagus	5.33	60	8	+	GNO	+	GNO	−	GNO	−	GNO	−	GNO	−	GNO	++
Kale	5.27	58	GNO	++	GNO	−	GNO	−	GNO	−	GNO	−	GNO	−	GNO	−

[a] Growth and toxin production were determined with vials containing 10 ml of cooked puréed vegetables under anaerobic conditions. Each vial was inoculated with ca. 10^3 spores of the indicated test strain per ml.
[b] TVG, time to visible growth (days); GNO, growth not observed.
[c] +, toxin detected in the ELISA; ++, toxin detected in the ELISA and in the mouse test; +*, toxin detected in the ELISA but not in the mouse test; −, toxin not detected in the ELISA. Note that there was no toxin in cooked potatoes with any of the strains.
[d] Total incubation time was 60 days for strain Hazen 36208.

Effect of temperature on the growth of nonproteolytic *C. botulinum* type B in cooked puréed potatoes, mushrooms, and cauliflower. The effect of temperature on growth curves of nonproteolytic *C. botulinum* type B was tested in cooked vegetables prepared as described above, except that they were cooled under a headspace of nitrogen and 100-ml volumes were distributed into 150-ml bottles in an anaerobic cabinet (Don Whitley Scientific, Leeds, United Kingdom) filled with oxygen-free H_2-CO_2-N_2 (10:5:85 [vol/vol]) before sterilization at 121°C for 15 min. The pHs of the cooked vegetables were 5.71 in potatoes, 6.29 in mushrooms, and 5.56 in cauliflower (each was the average of duplicate measurements). The water activity of cooked puréed vegetables was measured with a Decagon Aqualab CX2 dew point water activity meter (GBX SARL, Romans-sur-Isère, France) and was 0.99 for each vegetable purée (each was the average of four replicate samples). A suspension was prepared which contained equal numbers of spores of six strains of nonproteolytic *C. botulinum* type B (Eklund 2B, Eklund 17B, Hobbs FT50, Colworth 151, 2129B, and 4672U-1) and had a final concentration of 10^6 spores per ml. A 100-μl sample of this suspension was used to inoculate each of the 100-ml vials of cooked puréed potatoes, mushrooms, and cauliflower to give a final concentration of 10^3 spores per ml. Test vials (one vial per temperature and per vegetable, precooled before inoculation) were then transferred to low-temperature incubators. Temperatures were recorded every 15 min throughout the incubation period (17). Target temperatures were 16, 10, 8, and 5°C. For more than 98.8% of the time, the temperatures were lower than the target temperature + 1.0°C. Viable counts were determined at inoculation and then at appropriate intervals during incubation. Inoculated vials were observed for production of gas bubbles at each sampling time. A 1-ml sample was removed to determine the viable count and replaced by a volume of oxygen-free N_2-H_2 (90:10 [vol/vol]). Counts were made by preparing 10-fold dilutions in PYGS (25) under a flow of oxygen-free N_2-H_2 (90:10 [vol/vol]) and spreading 100-μl samples on duplicate plates of VL blood agar (17). The spread plates were transferred within 10 min to anaerobic jars under a headspace of H_2-CO_2 (90:10 [vol/vol]) and incubated for 48 h at 30°C. At each sampling time, a further sample was removed, diluted 10-fold, and kept frozen at −18°C for up to 8 weeks prior to analysis for toxin.

Significant growth of nonproteolytic *C. botulinum* type B occurred in cooked puréed potatoes at 16 and 8°C and in cooked mushrooms and cooked cauliflower at 16, 10, 8, and 5°C. No growth was detected in cooked potatoes at 10 and 5°C after 27 and 38 days of incubation, respectively. Curves were fitted to the growth data with the Baranyi model (4) to derive doubling time, lag time, and time to a 1,000-fold increase. Fitted growth curves are shown for mushrooms (Fig. 1). At each incubation temperature, time to a 1,000-fold increase was more rapid in mushrooms than in cauliflower, and that in cauliflower was more rapid than that in potatoes (Table 2). Time to toxin was taken as the first sampling time at which an A_{492} higher than 0.5 was obtained when 10-fold-diluted samples were tested in the ELISA. This is probably equivalent to more than 50 50% mouse lethal doses of type B toxin per ml (37). Similar results were obtained when time to visible growth and time to toxin were measured (Table 2) and when individual strains were tested (Table 1). Toxin was detected within 3 to 5 days at 16°C, 11 to 13 days at 10°C, 10 to 34 days at 8°C,

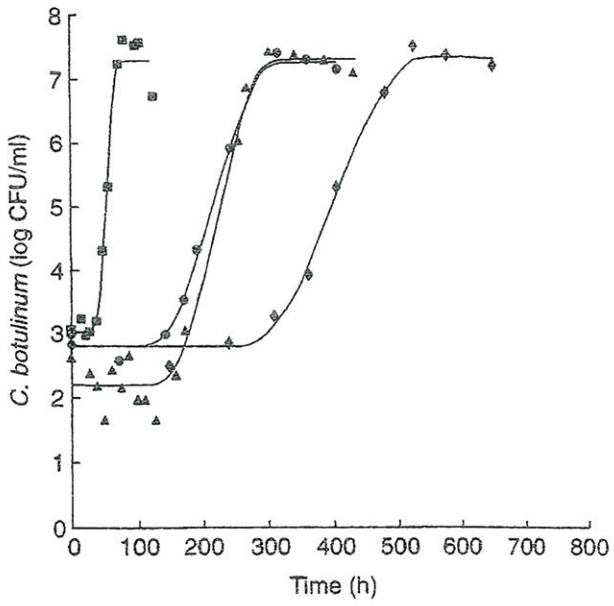

FIG. 1. Effects of incubation temperature on the growth from spores of a mixture of strains of nonproteolytic *C. botulinum* type B in cooked puréed mushrooms at pH 6.29. Points are actual counts at 16°C (■), 10°C (▲), 8°C (◆), and 5°C (◆). The lines show the fitting of the counts to the Baranyi equation.

TABLE 2. Growth of and toxin production by nonproteolytic *C. botulinum* type B in vials of cooked puréed vegetables at temperatures between 5 and 16°C

Vegetable	Target temp (°C)[a]	Calculated time[b]			RMSE[c]	Observed time		Toxin in the mouse test
		Doubling (h)	Lag (h)	To 1,000-fold increase (days)		To visible growth (days)	To toxin (days)[d]	
Potato	16	2.6	83	4.7	0.63	4.5	5.3	NT[e]
Mushroom	16	2.1	38	2.6	0.22	2.9	2.9	NT
Cauliflower	16	3.0	52	3.4	0.16	3.9	3.9	NT
Mushroom	10	7.0	161	9.6	0.63	10.5	10.5	+
Cauliflower	10	12.6	235	15.0	0.43	13.1	13.1	+
Potato	8	10.3	628	30.4	0.29	31.0	33.9	+
Mushroom	8	8.9	146	9.8	0.37	7.1	10.2	+
Cauliflower	8	11.3	288	16.8	0.39	14.9	17.1	+
Mushroom	5	12.4	304	17.8	0.43	20.0	20.0	+
Cauliflower	5	8.5	383	19.6	0.62	21.0	19.0	+

[a] There was no growth in cooked potatoes at 10 and 5°C.
[b] Growth parameters (doubling time, lag time, and time to a 1,000-fold increase) were estimated with the Baranyi equation (4).
[c] RMSE, root mean square error of fit of curve to data.
[d] First incubation time with $A_{492} > 0.5$ of ELISA reading in 10-fold-diluted samples. The A_{492} of diluted samples on inoculation was <0.1.
[e] NT, not tested.

and 17 to 20 days at 5°C. The presence of botulinum neurotoxin in samples was confirmed with the mouse test (Table 2).

The doubling times of nonproteolytic *C. botulinum* type B in cooked vegetables at 16, 10, 8, and 5°C were generally shorter than those observed in laboratory medium under optimum conditions (16, 17, 32, 33). When exact incubation temperature, pH, and NaCl concentration were input into a predictive model for nonproteolytic *C. botulinum* developed in this laboratory (17) and available through Food MicroModel (Food MicroModel, Ltd., Leatherhead, Surrey, United Kingdom), the predicted doubling times, lag times, and time to a 1,000-fold increase in culture medium were shorter (often marginally) than those observed in cooked vegetables.

The earliest times to toxin detection in other foods have generally ranged from 1 to 4 days at 30°C, from 6 to 20 days at 8 to 10°C, and from 14 to 30 days at 4 to 6°C (1, 3, 6, 7, 9–14, 19–21, 23, 24, 30, 36, 38, 41). The earliest time to toxin detection in vegetables is within this range, albeit toward the longer end. From these findings, it can be concluded that the potential for growth of and toxin production by nonproteolytic *C. botulinum* is as high in some cooked vegetables as in fish, meat, or poultry.

Conclusion. This work has shown that many vegetables supported growth and toxin production by nonproteolytic *C. botulinum* at refrigeration temperatures and that the time to toxin production is within the range reported for other food groups (e.g., meat, fish, and poultry). Consequently, recommendations and guidelines proposed to control the growth of nonproteolytic *C. botulinum* and ensure the safety of REPFEDs (2, 26) apply to REPFEDs made of or containing vegetables. Recommendations include a short shelf life for products that rely only on refrigeration temperature for preservation, with additional preservative factors (heat treatment, lowered pH, and/or lowered water activity) necessary for products with an extended shelf life.

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REFERENCES

1. **Abrahamsson, K., B. Gullmar, and N. Molin.** 1966. The effect of temperature on toxin formation and toxin stability of *Clostridium botulinum* type E in different environments. Can. J. Microbiol. 12:385–394.
2. **Advisory Committee on the Microbiological Safety of Food, U.K.** 1992. Report on vacuum packaging and associated processes. Her Majesty's Stationery Office, London.
3. **Baker, D. A., C. Genigeorgis, J. Glover, and V. Razavilar.** 1990. Growth and toxigenesis of *C. botulinum* type E in fishes packaged under modified atmospheres. Int. J. Food Microbiol. 10:269–290.
4. **Baranyi, J., and T. A. Roberts.** 1994. A dynamic approach to predicting bacterial growth in foods. Int. J. Food Microbiol. 23:277–294.
5. **Baumgart, J.** 1987. Vorkommen und Vermehrung von *Clostridium botulinum* in vakuumverpakten rohen und pasteurisierten Kartoffeln und im Kartoffelsalat. Chem. Mikrobiol. Technol. Lebensm. 11:74–80.
6. **Cann, D. C., and L. Y. Taylor.** 1979. The control of the botulism hazard in hot-smoked trout and mackerel. J. Food Technol. 14:123–129.
7. **Cann, D. C., B. B. Wilson, G. Hobbs, and J. M. Shewan.** 1965. The growth and toxin production of *Clostridium botulinum* type E in certain vacuum packed fish. J. Appl. Bacteriol. 28:431–436.
8. **Carlin, F., and M. W. Peck.** 1995. Growth and toxin production by nonproteolytic and proteolytic *Clostridium botulinum* in cooked vegetables. Lett. Appl. Microbiol. 20:152–156.
9. **Eklund, M. W., F. T. Poysky, and D. I. Wieler.** 1967. Characteristics of *Clostridium botulinum* type F isolated from the Pacific Coast of the United States. Appl. Microbiol. 15:1316–1323.
10. **Eklund, M. W., D. I. Wieler, and F. T. Poysky.** 1967. Outgrowth and toxin production of nonproteolytic type B *Clostridium botulinum* at 3.3 to 5.6 C. J. Bacteriol. 93:1461–1462.
11. **Garcia, G., and C. Genigeorgis.** 1987. Quantitative evaluation of *Clostridium botulinum* nonproteolytic types B, E, and F growth risk in fresh salmon tissue homogenates stored under modified atmospheres. J. Food Prot. 50:390–397.
12. **Garcia, G. W., C. A. Genigeorgis, and S. Lindroth.** 1987. Risk of growth and toxin production by *Clostridium botulinum* nonproteolytic types B, E, and F in salmon fillets stored under modified atmospheres at low and abused temperatures. J. Food Prot. 50:330–336.
13. **Garren, D. M., M. A. Harrison, and Y.-W. Huang.** 1994. *Clostridium botulinum* type E outgrowth and toxin production in vacuum-skin packaged shrimp. Food Microbiol. 11:467–472.
14. **Genigeorgis, C. A., J. Meng, and D. A. Baker.** 1991. Behavior of nonproteolytic *Clostridium botulinum* type B and E spores in cooked turkey and modeling lag phase and probability of toxigenesis. J. Food Sci. 56:373–379.
15. **Gola, S., and S. Mannino.** 1985. Produzione di tossina in substrato colturale e alimentare da parte di alcuni ceppi di *Clostridium botulinum*. Ind. Conserve 60:39–41.
16. **Graham, A., and B. M. Lund.** 1993. The effect of temperature on growth of non-proteolytic type B *Clostridium botulinum*. Lett. Appl. Microbiol. 16:158–160.
17. **Graham, A. F., D. R. Mason, and M. W. Peck.** A predictive model of the effect of temperature, pH and sodium chloride on growth from spores of non-proteolytic *Clostridium botulinum*. Int. J. Food Microbiol., in press.
18. **Hatheway, C. L.** 1993. *Clostridium botulinum* and other clostridia that produce botulinum neurotoxin, p. 3–20. *In* A. H. W. Hauschild and K. L. Dodds

(ed.), *Clostridium botulinum*: ecology and control in foods. Marcel Dekker, New York.
19. **Huss, H. H., I. Schaeffer, A. Pedersen, and A. Jepsen.** 1979. Toxin production by *Clostridium botulinum* type E in smoked fish in relation to the measured oxidation reduction potential (Eh), packaging method and the associated microflora, p. 476–479. *In* J. J. Connel (ed.), Advances in fish science and technology. Fishing News Books Ltd., Farnham, United Kingdom.
20. **Huss, H. H., I. Schaeffer, E. R. Petersen, and B. C. Cann.** 1979. Toxin production by *Clostridium botulinum* type E in fresh herring in relation to the measured oxidation reduction potential (Eh). Nord. Vet.-Med. 31:81–86.
21. **Ikawa, J. Y., and C. Genigeorgis.** 1987. Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rockfish fillets stored under modified atmospheres. Int. J. Food Microbiol. 4:167–181.
22. **Jensen, M. J., C. Genigeorgis, and S. Lindroth.** 1987. Probability of growth of *Clostridium botulinum* as affected by strain, cell and serologic type, inoculum size and temperature and time of incubation in a model broth system. J. Food Safety 8:109–126.
23. **Lerke, P.** 1973. Evaluation of potential risk of botulism from seafood cocktails. Appl. Microbiol. 25:807–810.
24. **Lindroth, S. E., and C. A. Genigeorgis.** 1986. Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rockfish stored under modified atmospheres. Int. J. Food Microbiol. 3:167–181.
25. **Lund, B. M., A. F. Graham, S. M. George, and D. Brown.** 1990. The combined effect of incubation temperature, pH and sorbic acid on the probability of growth of non-proteolytic type B *Clostridium botulinum*. J. Appl. Bacteriol. 69:481–492.
26. **Lund, B. M., and S. H. W. Notermans.** 1993. Potential hazards associated with REPFEDs, p. 279–303. *In* A. H. W. Hauschild and K. L. Dodds (ed.), *Clostridium botulinum*: ecology and control in foods. Marcel Dekker, New York.
27. **Lund, B. M., and M. W. Peck.** 1994. Heat-resistance and recovery of non-proteolytic *Clostridium botulinum* in relation to refrigerated, processed foods with an extended shelf-life. J. Appl. Bacteriol. Symp. Suppl. 76:115S–128S.
28. **Lund, B. M., G. M. Wyatt, and A. F. Graham.** 1985. The combined effect of low temperature and low pH on survival of, and growth and toxin formation from, spores of *Clostridium botulinum*. Food Microbiol. 2:135–145.
29. **Meng, J., and C. A. Genigeorgis.** 1993. Modeling lag phase of nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. Int. J. Food Microbiol. 19:109–122.
30. **Meng, J., and C. A. Genigeorgis.** 1994. Delaying toxigenesis of *Clostridium botulinum* by sodium lactate in "Sous-vide" products. Lett. Appl. Microbiol. 19:20–23.
31. **Notermans, S., J. Dufrenne, and J. H. Keijbets.** 1981. Vacuum-packed cooked potatoes: toxin production by *Clostridium botulinum* and shelf life. J. Food Prot. 44:572–575.
32. **Ohye, D. F., J. H. B. Christian, and W. J. Scott.** 1966. Influence of temperature on the water relations of growth of *Cl. botulinum* type E, p. 136–143. *In* M. Ingram and T. A. Roberts (ed.), Botulism 1966. Proceedings of the Fifth International Symposium on Food Microbiology, Moscow 1966. Chapman and Hall, London.
33. **Ohye, D. F., and W. J. Scott.** 1957. Studies on the physiology of *Clostridium botulinum* type E. Aust. J. Biol. Sci. 10:85–94.
34. **Peck, M. W., D. A. Fairbairn, and B. M. Lund.** 1992. The effect of recovery medium on the estimated heat-inactivation of spores of non-proteolytic *Clostridium botulinum*. Lett. Appl. Microbiol. 15:146–151.
35. **Peck, M. W., B. M. Lund, D. A. Fairbairn, A. S. Kaspersson, and P. C. Undeland.** 1995. Effect of heat treatment on survival of, and growth from, spores of nonproteolytic *Clostridium botulinum* at refrigeration temperatures. Appl. Environ. Microbiol. 61:1780–1785.
36. **Post, L. S., D. A. Lee, M. Solberg, D. Furgang, J. Specchio, and C. Graham.** 1985. Development of botulinal toxin and sensory deterioration during storage of vacuum and modified atmosphere packaged fish fillets. J. Food Sci. 50:990–996.
37. **Potter, M. D., J. Meng, and P. Kimsey.** 1993. An ELISA for detection of botulinal toxin types A, B, and E in inoculated food samples. J. Food Prot. 56:856–861.
38. **Read, R. B., Jr., J. G. Bradshaw, and D. W. Francis.** 1970. Growth and toxin production of *Clostridium botulinum* type E in milk. J. Dairy Sci. 53:1183–1186.
39. **Solomon, H. M., D. A. Kautter, and R. K. Lynt.** 1982. Effect of low temperatures on growth of nonproteolytic *Clostridium botulinum* types B and F and proteolytic type G in crabmeat and broth. J. Food Prot. 45:516–518.
40. **Solomon, H. M., R. K. Lynt, T. Lilly, Jr., and D. A. Kautter.** 1977. Effect of low temperatures on growth of *Clostridium botulinum* spores in meat of the blue crab. J. Food Prot. 40:5–7.
41. **Taylor, L. Y., D. D. Cann, and B. J. Welch.** 1990. Antibotulinal properties of nisin in fresh fish packaged in an atmosphere of carbon dioxide. J. Food Prot. 53:953–957.